Formulations. Formulations of the compounds of this invention are prepared for storage or administration by mixing the compound having a desired degree of purity with physiologically acceptable carriers. excipients, stabilizers etc., and may be provided in sustained release or timed release formulations. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical field, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co., (A.R. Gennaro edit. 1985). Such materials are nontoxic to the recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, acetate and other organic acid salts, antioxidants such as ascorbic acid, low molecular weight (less than about ten residues) peptides such as polyarginine, proteins, such as serum albumin, gelatin, or immunoglobulins, hydrophilic polymers such as polyvinylpyrrolidinone, amino acids such as glycine, glutamic acid, aspartic acid, or arginine, monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose or dextrins, chelating agents such as EDTA, sugar alcohols such as mannitol or sorbitol, counterions such as sodium and/or nonionic surfactants such as Tween, Pluronics or polyethyleneglycol.

Without further description, it is believed that one of ordinary skill in the art, using the preceding description and the following illustrative examples, can make and utilize the compounds of the present invention and practice the claimed methods.

EXAMPLES

The following working examples which disclose effects of thapsigargin treatment *in vitro* and *in vivo* in cell lines and in a mouse model of cystic fibrosis specifically point out certain embodiments of the present invention. These examples are not to be construed as limiting in any way the scope of the invention. Other examples involving ER chaperone and UGGT regulation as well as other proteins that regulate intracellular targeting of mis-folded proteins will be apparent to the skilled artisan. Assays analogous to those described below can be utilized in examining other agents that regulate UGGT or other proteins that regulate mis-folded proteins.

Tissue culture/ Cell lines

IB3-1 (Zeitlin et al., 1991) and ΣCFBE290$^-$ (Kunzelman *et al.*, Am. J. Resp. Cell. Mol. Biol. 8:522-529.(1993)) cells are CF-affected airway epithelial cell lines. Both IB3-1 and ΣCFBE290$^-$ are immortalized, well-characterized human bronchial epithelial cell lines

derived from CF-patients. The cell lines retain the diagnostic feature of CF-affected epithelial cells: a lack of cAMP-stimulated, PKA-activated Cl^- channel activity. Genotypically, IB3-1 is a compound heterozygote containing the ΔF508 mutation and W1282X, a nonsense mutation with a premature termination signal. The W1282X mutation does not result in a stable mRNA and yields no protein (Hamosh *et al.*, Hum. Mol. Gen. 1:542-544.(1992)). Therefore, the only stable CFTR protein produced in the IB3-1 cells is the ΔF508 product.

The ΣCFBE290$^-$ cell line is derived from a patient homozygous for the ΔF508 mutation. Both cell lines were grown at 37° in 5% CO^2. The IB3-1 cells were maintained in LHC-8 media (Biofluids) supplemented with 5% fetal calf serum, tobramycin (20 ug/ml), penicillin (100 U/ml), streptomycin (100 ug/ml). The ΣCFBE290$^-$ cells were maintained in Dulbucco's Modified Eagles medium (DMEM) supplemented with 10% fetal calf serum, tobramycin (20 ug/ml), penicillin (100 U/ml), and streptomycin (100 ug/ml).

The CFPAC-1 cell line is a ductal pancreatic adenocarcinoma cell line derived by differential trypsinization of explant cultures from a metastatic lesion in the liver of a 26 year old male with CF (Schoumacher *et al.*, Proc. Natl. Acad. Sci. 87:4012-4016 (1990)). The cell line is homozygous for expression of ΔF508 CFTR and has the ion transport properties of CF-affected epithelia. CFPAC-1 cells show epithelial morphology and polarization with apical microvilli.

CFPAC cells were grown at 37° in 5% CO^2 and maintained in Isocove's modified Dulbucco's medium supplemented with 10% fetal calf serum. Both for measurements of short circuit current and for immunofluorescence experiments, these cells were grown on collagen coated permeable supports (Transwell Snapwell filter cups, Corning Costar, Cambridge, MA). The well characterized T84 intestinal epithelial cell line was grown according to standard methods (Cohn *et al.*, Proc. Nat. Acad. Sci. 89:2340-2344 (1992); Bell and Quinton, Am. J. Physiol. 262:C555-C562.(1992)) and were also plated on permeable supports for short circuit current assays.

Experiment 1. Patch clamp analysis.

Materials and Methods. Single channel patch clamp studies were performed using conventional procedures on the CF-affected bronchial epithelial cell lines, IB3-1 and ΣCFBE290$^-$ (Egan *et al.*, Am. J. Physiol. 268:C243-C251 (1995)). Cells were grown in culture flasks on glass chips coated with collagen (150 ug/ml), fibronectin (10 ug/ml), and bovine serum albumin (10 ug/ml).

When cells were at 75% confluence they were incubated with 1uM thapsigargin (or vehicle alone) for 1.5 hours at 37°C using the following protocol. First, the LHC-8 media or DMEM was removed from the tissue culture dish and the cells were rinsed with phosphate buffered saline. Fresh LHC-8 media containing 1uM thapsigargin was added to the cell culture dish. After the 1.5 hour thapsigargin exposure, cells were rinsed with fresh media and allowed to incubate for 2 hours at 37°C prior to patch clamping. The patch clamp bath solution contained (in mM) 150 NaCl, $2MgCl_2$, 1 EGTA, 5 HEPES, and 0.5 $CaCl_2$, pH=7.3. The pipette solution contained (in mM) 150 NaCl, 2 $MgCl_2$, 5 HEPES, and 2 $CaCl_2$, pH=7.3.

Patch clamp studies were performed at 22-25°C. Data were amplified on an Axopatch 200A patch clamp amplifier and recorded on videotape for later analysis. Data were low pass filtered and digitized at 1kHz. Data were analyzed using Pclamp6.

Results. The surface expression of ΔF508 CFTR was initially examined by patch clamp analysis performed on two different treated and untreated CF-affected respiratory epithelial cell lines, IB3-1 (Zeitlin *et al.*, Am. J. Resp. Cell. Mol. Biol. 4:313-319 (1991)) and ΣCFBE290⁻ (Kunzelman *et al.*, Am. J. Resp. Cell. Mol. Biol. 8:522-529 (1993)).

In the untreated CF-affected cells, no low conductance chloride channels could be activated with a cAMP-stimulation cocktail containing IBMX and forskolin (Figure 1A). These findings are consistent with the primary CF defect. In contrast, treatment with thapsigargin dramatically enhanced the IB3-1 and ΣCFBE290⁻ cells' chloride conductance.

Cells were incubated in 1 μM thapsigargin for 90 minutes, after which they were incubated for 2 hours in the absence of the drug. Patch clamp analysis of the treated cells revealed that their plasma membranes now contained abundant low conductance chloride channel activity (Figure 1B and Table 1). The biophysical characteristics of the channel activity were consistent with those of the channel formed by the ΔF508 CFTR protein (Dalemans *et al.*, Nature 354:526-528 (1991); Egan *et al.*, Am. J. Physiol. 268:C243-C251 (1995); Rubenstein *et al.*, J. Clin. Invest. 100:2457-2465 (1997); Haws *et al.*, Am. J. Physiol. 270:C1544-C1555.(1996); Hwang *et al.*, Am J Physiol. 273:C988-998 (1997)). Thus, the current versus voltage relationship is linear (Figure 2A), revealing an average single channel conductance of 11.8 pS. Furthermore, analysis of an open state histogram (Figure 2B) produces a calculated P_o of 0.12. Channel activity could be inhibited by glibenclamide (data not shown). The levels of